

(U)

K J

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

2

AD-A209 650 IC

1b. RESTRICTIVE MARKINGS

N/A

3. DISTRIBUTION / AVAILABILITY OF REPORT

Distribution unlimited

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE

N/A

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

N/A

5. MONITORING ORGANIZATION REPORT NUMBER(S)

N/A

6a. NAME OF PERFORMING ORGANIZATION
Univ. of California, Santa
Barbara, Marine Science Inst.6b. OFFICE SYMBOL
(If applicable)
N/A

7a. NAME OF MONITORING ORGANIZATION

Office of Naval Research

6c. ADDRESS (City, State, and ZIP Code)

Marine Science Institute
University of California
Santa Barbara, CA 93106

7b. ADDRESS (City, State, and ZIP Code)

800 N. Quincy Street
Arlington, VA 22217-50008a. NAME OF FUNDING / SPONSORING
ORGANIZATION

Office of Naval Research

8b. OFFICE SYMBOL
(If applicable)

ONR

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

N00014-88-K-0060

8c. ADDRESS (City, State, and ZIP Code)

800 N. Quincy Street
Arlington, VA 22217-5000

10. SOURCE OF FUNDING NUMBERS

PROGRAM
ELEMENT NO.PROJECT
NO.TASK
NO.WORK UNIT
ACCESSION NO.

1141MB

4412028

11. TITLE (Include Security Classification)

Molecular Biology of the Photoregulation of Photosynthetic Light - Harvesting Complexes
in Marine Dinoflagellates (U)

12. PERSONAL AUTHOR(S)

Barbara B. Prezelin, Edward L. Triplett

13a. TYPE OF REPORT

Annual

13b. TIME COVERED

FROM 6/15/88 TO 6/15/89

14. DATE OF REPORT (Year, Month, Day)

890602

15. PAGE COUNT

13

16. SUPPLEMENTARY NOTATION

N/A

17. COSATI CODES

FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Dinoflagellates, gene regulation, light harvesting complexes
(LHC), peridinin-chlorophyll a-protein (PCP), transcriptional
controls, translational controls, DNA organization, (Cont'd)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Our goal is to continue to use biotechnological techniques to study the genetic bases of light- and nutrient- regulation of photosynthetic light-harvesting complexes in marine dinoflagellates and their consequences for the bio-optical features of these algae. The studies are significant to determining the molecular bases of environmental regulation of gene expression and photosynthetic performance in marine phytoplankton. Results also contribute to understanding the linkages between light environments and cell optical properties, key elements in physiologically-based bio-optical models being developed to predict ocean primary production.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT

☒ UNCLASSIFIED/UNLIMITED
 ☐ SAME AS RPT.
 ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION

(U)

22a. NAME OF RESPONSIBLE INDIVIDUAL
Michael T. Marron22b. TELEPHONE (Include Area Code)
(202) 696-476022c. OFFICE SYMBOL
ONR

89 6 26 070

(U)

SECURITY CLASSIFICATION OF THIS PAGE

18. (Continued) cloning of cDNA for PCP, cloning of genomic DNA for PCP, photosynthesis.

(U)

SECURITY CLASSIFICATION OF THIS PAGE

ANNUAL REPORT
ONR Contract # N00014-88-K-0060
88 June 15 through 89 June 15

Molecular Biology of the Photoregulation of Photosynthetic Light-Harvesting Complexes in Marine Dinoflagellates

Barbara Prezelin and Edward Triplett
Marine Science Institute
University of California Santa Barbara

Project Summary

Our long term objective is to study the genetic basis of light- and nutrient- regulation of photosynthetic light harvesting complexes in marine dinoflagellates.. The peridinin-chorophyll a-protein (PCP) complexes of Glenodinium sp. are being developed as a model system for the proposed genetic analyses. The specific aims of the present study are: 1) To characterize PCP apoprotein cDNA selected from Lgt10 and Lgt11 cDNA libraries; 2) To characterize PCP genes from an Embl 3 library and from genomic Lgt10 clones. This will include complete sequence analysis, positioning of intervening sequences and determination of upstream sequences likely to be involved as control regions responsive to wavelength and intensity of light. As the transcriptional/translational levels of control of PCP gene(s) expression by light history and nutrient availability are determined, clues to the nature of the photoreceptor involved may be deduced and provide direction for studies aimed at identifying the photoreceptor and/or its mode of light-regulation of PCP turnover; (3) To determine the interactive influence that nutrient availability and irradiance may have on the kinetics of PCP transcription and/or translation; (4) To assess the linkages between light dependent gene regulation of photosynthesis and the optical biology of the whole cell; and (5) To explain the multiplicity of isoelectric forms of the PCP light-harvesting complexes.

Results to date

To date, the following information regarding the molecular genetics of the test dinoflagellate, Glenodinium sp. has been gained through the efforts of our research group. The major contributor to each part of the project is indicated in parentheses.

i) We have produced and characterized highly specific polyclonal PCP antibodies (see appendices 1,4,5). The antibodies have been purified by affinity chromatography using SDS gel purified, agarose bead bound PCP apoprotein as ligand. Affinity purified antibody reacts only with PCP apoprotein as judged by Western blots of crude Glenodinium sp. protein extracts. Antibody reacts with all isoelectric



odes

for
Special

A-1

forms of PCP as judged by western blots of isoelectric focusing acrylamide gels (Roman).

ii) The Glenodinium sp. affinity purified antibodies have been used to perform a comparative immunological study. (see appendix 7) These antibodies are not only highly specific for the various conformers of PCP apoprotein, but also react strongly and specifically with PCP complexes from other dinoflagellates. The cross reactivity of PCP antibodies on western occurred whether the PCP apoprotein was 1) the larger 35kD form observed in the PCP complexes of Gonyaulax polyedra and the clones of Symbiodinium spp. isolated from Rhodactis sancti-thomae (anemone), Ragactis lucida (anemone), Monastrea cavernosa (coral), and a Bartholemea sp. (anemone) or 2) the smaller 15kD form or apoprotein found in PCP complexes of Glenodinium sp., Amphidinium carterae and clones of Symbiodinium spp. isolated from Condylactis gigantea (anemone) and Tridacna maxima (clam). A preparation Symbiodinium sp. from Anthopleura elegantissima (anemone) was unique in containing both a 35kD and a 15kD apoprotein reacting with anti-PCP antibodies. Antibodies do not react with fucoxanthin containing light harvesting complexes. These results demonstrate the usefulness of these antibodies as a broad spectrum tool for use in the detection of and eventual quantification of PCP apoproteins from a wide variety of PCP containing dinoflagellates.

iii) Glenodinium Poly A+ RNA was used to construct two cDNA libraries, one in Lambda gt10 and the other in Lambda gt11. The gt11 library was screened with affinity purified PCP antibody using a biotinylated goat anti-rabbit antibody and avidin horse radish peroxidase for visualization. Several clones were recovered, one of which was sequenced. This 198bp fragment was nick translated and used to screen the gt10 library. Screening 10^5 pfu resulted in the recovery of eight recombinant PCP cDNA clones ranging in size from about 0.8 to 0.2 kb. Restriction patterns of the clones indicate that they represent at least five different genes. The largest insert has been completely sequenced. The sequence contains an open reading frame sufficiently large to code for a 15.5 K Dalton protein (the size of processed PCP apoprotein). Kyle-Doolittle and Goldman plots indicate two hydrophobic pockets at inferred amino acid residues 25-45 and 55-75. These are of the right configuration to supply a pocket for the accommodation of the pigments. The inferred amino sequence of the rest of the molecule is extremely polar as would be expected of the PCP light harvesting complex (it is soluble in 75 % saturated ammonium sulfate). The inferred amino acid sequence confirms our direct observations that there are no glycosylation sites for this molecule and it is therefore devoid of carbohydrate (see appendix 3) (Nadathur and Triplett).

To confirm the identity of PCP cDNA we have performed hybrid release and translation using the sequenced cDNA as a bound probe to hybridize with total Poly A+ RNA. Material hybridized to this probe at high stringency was translated

in a reticulocyte system and produced a product, all of which could be identified immunologically on western blots as PCP apoprotein. We are in the process of further confirming the identity of the cloned cDNA by comparison with an amino terminal sequence of amino acids which we have performed on the apoprotein. The sequence is NH₂-asp-glu-ilu-gly-asp-ala-ala-lys-lys-leu-gly-asp-asp-ala-ser-tyr-ala phe-ala-lys-glu (Triplett).

We know from the hybrid release and translation experiments that PCP is translated as a preprotein of about 23kD. Our cloned and sequenced PCP cDNA does not contain this preprotein leader sequence, and we have therefore screened about 1.2×10^6 gt10 recombinant phage in search of inserts containing the 5' sequence describing the leader peptide. None were found. We are therefore obtaining the leader sequence by a different strategy described in the next section of this report.

iv) Glenodinium DNA has been characterized by renaturation kinetics, thermal denaturation and by fluorimetric determination of DNA per cell (See appendix 2). We obtained high molecular weight DNA from Glenodinium sp. by homogenization in liquid nitrogen, extraction in EDTA/sarcosyl/proteinase K and repeated extraction in phenol and chloroform (Blin and Stafford, 1976). We obtain excellent separation of chloroplast and main band DNA by two centrifugations to equilibrium on CsCl/ethidium bromide gradients. Renaturation kinetics were measured under conditions used by Britton and Kohne (1968) and monitored by an S1 nuclease technique (Harrison et al., 1974). About 10% of the DNA represented very highly repeated sequences, being renatured at a Cot time less than 3×10^{-4} mole \times sec L⁻¹. An additional 17.5% of the DNA was renatured over a wide range of copy number, with a Cot 1/2 of about 0.5 mole \times sec L⁻¹. The remaining majority of DNA (72.5%) represented single copies, having a Cot 1/2 of about 9×10^2 mole \times sec L⁻¹. The complexity of this largest fraction was calculated to be about 5×10^8 base pairs. Thermal denaturation profiles indicate that the moderately repeated fraction is a small family of sequences with relatively little sequence divergence. The data indicate that these sequences are arranged in large blocks of repeats which appear to be sparsely interspersed with low copy DNA sequences. The chemical measurement of DNA content yields a value of 9.10 pg/cell (Triplett).

v) A genomic library for Glenodinium sp. has been constructed using bacteriophage EMBL 3 as a vector. High molecular weight total DNA was partially digested with MboI to optimize for recovery of 20kb fragments, which were ligated into EMBL 3 which had been double cut with EcoRI and HindIII. Packaging and transformation were performed according to the protocol of the packaging mix manufacturers (Promega). Estimated recovery of recombinant phage is 4.3×10^5 pfu (Triplett).

vi) Genomic Southern blots have been probed with both the truncated and nearly full length cDNAs. The two probes light up

identical bands on EcoR1 digested DNA. Five major bands (4.5, 3.8, 3.6, 2.6 and 1.4 kbp) were observed. It is possible that each band represents a different gene since the smaller probe contains only the center of the translatable portion of the gene whereas the larger probe contains both ends of the translatable material (Nadathur).

vii) Each of the EcoR1 genomic fragments hybridizing to PCP cDNA has been cut out of a low melt agarose preparative gel, ligated into gt10 and plaque purified using PCP cDNA as a probe (Nadathur).

viii) We have performed preliminary experiments to determine steady state levels of PCP apoprotein and PCP mRNA under conditions of low and high irradiance so as to gain some insight into levels of genetic control in this system (see appendix 1). Western blots of the protein preparations were prepared using affinity purified PCP antibody. Peroxidase visualized bands were scanned at 550nm and the area under the PCP peak was obtained by integration. PCP levels at low irradiance were about 2.5 times higher than at low light. We do not know at this time whether this reflects differences in rates of accumulation or degradation of PCP (Roman).

Two types of experiments, involving either northern blots or cell free translation, were performed to gain information about PCP mRNA levels in the high and low light cultures. Exactly equivalent amounts of total RNA from low and high light cultures were electrophoresed in parallel on denaturing agarose gels, transferred to a nylon membrane and probed with nick translated PCP cDNA. Two RNA bands were visualized by autoradiography in each lane (0.8 and 1.4 kb.). Each band was cut out and counted. The 0.8 kb band was about three fold amplified in low light as compared to high light material. The 1.4 kb band had identical counts in low and high light derived RNA. These data are consistent with the idea that there are two classes of PCP genes, one constitutive and the other regulated by light either through control of transcription or stability of mRNA. Exactly equivalent amounts of poly A+ RNA from low and high light cultures were translated in a reticulocyte cell free system. Aliquots of each were immunoprecipitated and electrophoresed on SDS gels in parallel with total translational product. Immunoprecipitable products are obviously more abundant in the low light system. Most bands visualized in total translational product are not different in high or low light, although several unidentified bands were either higher or lower in frequency in low light cultures (see appendix 1). In addition, immunoprecipitated products were of a higher molecular weight than mature PCP apoprotein detected by Western blots. This indicates that PCP apoproteins are synthesized with a leader peptide sequence which is presumably processed during their transport to the chloroplast. This processing would not be expected to occur in

an in vitro, heterologous translation system (Nadathur and Roman).

ix) We have developed an ELISA method to quantify PCP protein after light dependent regulation of PCP expression. This method allows us to distinguish quantitatively between different isoelectric variants of the protein. We have discovered that microtiter wells pretreated with polyglutamate will selectively bind more basic PCP conformers, whereas wells pretreated with polylysine bind more acidic conformers at neutral pH. Coating of wells with both ligands allows for binding of total PCP. Wells prepared in this way are treated with PCP affinity purified antibody followed by protein-A horseradish peroxidase conjugates. Wells are developed with a peroxidase substrate, and protein is quantified by absorbancy at 405 nm (Biorad ELISA plate reader)(Jovine).

Plans for the next year

Glenodinium sp. will be the primary test organism in all studies designed to determine the genetic bases for the light-regulated turnover of the major light-harvesting complex PCP in dinoflagellates and the consequences for the optical biology of the organism. The molecular topology, functional organization and photophysiology of PCP have been most clearly detailed in Glenodinium sp. (cf. Prezelin, 1981, 1987; Boczar and Prezelin, 1986) and excellent progress has been made in the last year toward generating the necessary tools to study its molecular biology. Listed below are the specific objectives for the proposed studies for the next 2 years, a summary of the approaches to be taken, an outline of the variety of molecular, biochemical and physiological measures to be employed, comment on the expected significance of experimental design (In each case the major contributors to each of the objectives will be mentioned in parentheses).

Objective 1: to sequence the mRNA coding region for the amino terminal peptide (Triplet).

To date we have isolated cDNA clones of varying sizes from lambda gt11 and lambda gt10 libraries, the largest being 730 bp. Results from our experiments with in vitro translation of mRNA as well as Western blots from protein preparations indicate that the PCP apoprotein has a leader peptide which is processed presumably during transport into the chloroplast (see appendix 1). The pre-protein has an apparent molecular weight of 23 kD, while the mature apoprotein has a molecular weight of 15.5 kD. We estimate the coding sequence of the mature protein to be approximately 450 bp. While the 730 bp clone is large enough to encode the mature protein (as determined by sequence analysis), it does not include the sequence for the leader peptide.

In order to determine exactly the number of nucleotides missing from the 5'-end of our cloned cDNA we will first perform a primer extension experiment. Total poly A+ RNA is used as a

template and a synthetic 15 mer anti mRNA oligonucleotide complimentary residues 701 to 716 is used as a primer for reverse transcriptase mediated elongation. Following the reverse transcriptase reaction the single stranded cDNA representing the 5' end of the RNA is sized on a denaturing acrylamide sequencing gel. We expect to see two bands on the gel since we have seen two size classes of PCP mRNA on northern blots.

Since we have at least two different populations of PCP mRNA in total poly A+ RNA this material cannot be used as a template for sequencing the 5' leader region. Instead, we will use the genomic clones for PCP that we have isolated. Appropriate clones will be transferred from gt10 to pUC19 and the synthetic oligonucleotide described above will be used as a primer for sequencing by the dideoxy method. Intervening sequencing should be detectable by the presence of splice junctions, and the origin of the translated region should be rather close (in a 3' direction) to a TATA box (Triplet and Nadathur).

Objective 2: To characterize the PCP genes that we have isolated (Triplet).

By genomic Southern blotting of EcoRI digested Glenodinium DNA using nick translated full length or truncated PCP cDNA as a probe, we have identified five fragments with PCP homology. These fragments were ligated into gt10 and plaque purified. Each of these genomic DNA's are Southern blotted with small 5' fragments and 3' fragments of PCP cDNA to ensure that all of the translatable portions (with introns included) are present. Restriction maps of each genomic PCP DNA will be obtained (Berk and Sharp, 1977; Berk et.al., 1978). Appropriate restriction fragments will be cloned into plasmid pUC19 and sequenced. S1 nuclease mapping of the genes in conjunction with sequence data will be used to determine the start site of transcription and to identify upstream putative control sequences. By comparison to vascular plants we expect we will need to obtain PCP gene fragments containing at least one Kbp upstream from the transcription start site for our studies on control elements. If none of our clones contain such sequences we shall probe our Embl 3 genomic library until we find pieces of cloned DNA with sufficiently extensive upstream sequences.

We propose to analyze the control region of the PCP gene of Glenodinium sp. in an effort to identify any 5' upstream region that may be the target site, either directly or via a secondary messenger protein, of the photoregulation of PCP apoprotein transcription. Toward this end we are developing a plasmid vector can be introduced as a replicating unit into Glenodinium sp. and which also can be replicated and amplified as an autosome in E. coli. The general strategy for study of the PCP control region is to excise it from our cloned PCP gene and to ligate this fragment into the shuttle vector upstream from a gene which we can easily quantify (e.g.- chloramphenicol acetyl transferase) and which is placed under the transcriptional control of the PCP regulatory region. The recombinant vector

is then introduced into Glenodinium sp. and the transformed cells are tested for their ability to produce chloramphenicol acetyl transferase (CAT) in response to light perturbations. After optimal conditions of illumination for transcription of the CAT recombinant are established, the PCP regulatory region is modified with standard methods to define precisely the regions responsive to light.

The shuttle vector should be a) able to replicate in *E. coli*, yeast and Glenodinium sp. and b) selectable in all three organisms. The strategy we would use to isolate autonomously replicating sequences (ARS) of DNA from Glenodinium sp. is a modification of procedures of Revuelta and Jayaram (1986). Our initial experiments indicate the presence of Glenodinium sequences which are capable of functioning as ARS in yeast.

Once a plasmid containing the Glenodinium ARS sequence is isolated, a dominant selectable marker would be determined. The Kanamycin resistance gene is a selectable marker from the bacterial transposon Tn903 which encodes for amino-glycoside phosphotransferase-3' responsible for resistance to amino-glycoside antibiotics like neomycin, kanamycin and G-418. Initial experiments have shown the dinoflagellate to be sensitive to Kanamycin sulfate and we are in the process of constructing vectors which carry the Glenodinium ARS and Kanamycin gene from Tn90. If we fail to find ARS sequences capable of autonomous replication in Glenodinium sp., then we must transform cells by integration of test DNA into the host cell genome. In this case, the signal gene construct would be the same as described above. Using the approach of Paszkaowsky and Saul (1986), an effort would be made to electroporate dinoflagellate protoplasts (Adamich and Sweeney, 1976).

Objective 3: Patterns of transcription and translation of the PCP genes under different lighting conditions (Jovine, Triplett, Prezelin)

Light effects on PCP gene(s) transcription would be determined by comparing differences in PCP mRNA content in Glenodinium sp. log phase batch cultures maintained over a range of spectral irradiances. Probes to be used for quantifying mRNA under different physiological conditions are generated from our lambda gt11 library. PCP-encoding clones from the Embl3 library would be employed as gene specific probes to examine differential PCP gene expression under varying irradiance conditions. We would characterize the size and content of PCP gene(s) transcripts by DNA-RNA hybridization (Northern analysis). Poly-A tailed RNA would be isolated and electrophoresed in denaturing gels. Nitrocellulose blots of the gels would be hybridized to labelled DNA probes. Blots would be autoradiographed and transcript sizes. Quantification of PCP levels in Glenodinium sp. adapted to different irradiance levels would be examined by dot immunobinding assays for PCP apoproteins and by concurrent measurements of cellular peridinin

and Chl a content by high performance liquid chromatography (HPLC). Results should provide insight into the dependency of PCP gene(s) expression on wavelength and intensity in spectral environments representative of natural light fields where these microalgae thrive. Furthermore, a comparison of results from light-limited and light-saturated growth conditions should provide information on the influence growth status has on the spectral dependency of PCP photocontrol sites.

Objective 4: to determine the interactive influence that transitions in nutrient availability and irradiance may have on the kinetics of PCP gene(s) transcription and translation (Jovine, Triplett, Prezelin).

Transcriptional and possible translational control of PCP apoprotein synthesis would be examined by measurement of the rate of accumulation of transcripts in cells shifted from PCP-repressing to inducing conditions (ie. high light to low light). The possibility of translational control of PCP gene expression would be assessed by comparisons of these studies with those measuring apoprotein accumulation and turnover. If polypeptide and transcript accumulation appear synchronized, then PCP protein synthesis would appear to be primarily under transcriptional control. However, in the case that protein accumulation lags or increases relative to PCP mRNA accumulation, then PCP synthesis is not entirely controlled at the transcript level. Concurrent measurements of cellular peridinin and chl a content would provide comparative information on the kinetics of photocontrol of both the PCP chromophore and apoprotein biosynthesis. To determine the influence of nutrient depletion on the photocontrol of PCP gene expression, the above photoadaptation experiments would be repeated in stationary phase batch cultures.

Poly A+ RNA would be isolated from cells sampled at various time points during shifts from PCP-repressing to PCP-inducing conditions. The rate of PCP transcript accumulation would be measured by quantitative dot hybridization to respective labelled DNA PCP coding fragments and by in-vitro translation. Within a heterologous system, in-vitro translation detects the presence of functional RNA species. Quantitative dot blots measure both functional and other transcripts which may require further processing before translation or which may have been modified in-vivo to prevent translation. Populations would be labelled with 3H-leucine, since these autotrophic dinoflagellates are known to assimilate this amino acid from growth medium (Sweeney, pers. comm.). Samples would be taken at various time points following transfer from high to low light conditions, and the incorporated label quantified in whole cell extracts and through PCP immunoprecipitates of homogenized extracts. Samples would be quantified directly by scintillation counting or following SDS-PAGE, where protein bands of interest would be detected by fluorography, excised and the specific activity determined. The results would document the rate of PCP

apoprotein accumulation, as well as indicate the magnitude of PCP apoprotein enrichment over levels of radioactive total cellular protein. The turnover in a nonphotoregulated protein, such as actin would serve as a control in these studies.

Pulse-chase experiments would enable us to measure PCP apoprotein turnover in transiently photoadapting cells. This is particularly important if we are to distinguish between increases in PCP levels due to higher rates of synthesis as opposed to lower rates of degradation. Cultures would be briefly labelled with radioactive sulfate, resuspended in a medium of excess cold sulfate, and the turnover of the apoprotein monitored as the time-dependent change in radiolabel in PCP immunoprecipitated extracts and total protein. If there is indication that regulation at the level of translation is significant, then translational efficiency of PCP transcripts could be followed by measuring elongation rates and relative amounts of nascent PCP polypeptides associated with polysomes (Fan and Penman, 1970; Stewart-Blair, 1971; Mueckler et al., 1983).

Objective 5: to assess the linkages between light-dependent gene regulation of photosynthesis and the optical biology of the whole cell via concurrent determinations of influences of light history and nutrient availability on spectral absorption, fluorescence action spectra, quantum yield, and wavelength-dependent photosynthesis (Jovine, Nelson, Prezelin).

While aspects of the white light photophysiology of PCP-dependent photo-adaptation are fairly well detailed in dinoflagellates, the coordinated effect of irradiance on PCP turnover and resultant changes in the spectral signature, quantum yield and wavelength-dependent photosynthesis of the cell has been rarely documented. To determine these relationships between the photoregulation of the molecular genetics of PCP and the optical biology of photosynthesis at the whole cell level, a suite of bio-optical properties would be quantified at the same time that measurements of different levels of gene expression were made. Thus, as the site(s) light and nutrient regulation of PCP gene expression are being defined, we will also define the impacts on whole cell pigmentation and spectral signatures of light absorption, energy transduction efficiency, photosynthetic accommodations and quantum yield of carbon fixation. Such linkages will provide mechanistic insight and tests of developing bio-optical modes of phytoplankton productivity (cf. Smith et al. 1989).

Objective 6: Characterization of isoelectric forms of the PCP light harvesting complex (Jovine, Triplett).

DEAE-cellulose columns developed with a pH gradient reveal the presence of five conformers of the PCP light harvesting complex. Isoelectric focusing gels or preparative isoelectric focusing columns, on the other hand, indicate many more isoelectric variants. We are confident on the basis of data

discussed above that PCP is a multigene family, and this is reflected in the presence of different, distinguishable mRNA species. But this cannot account for the number of isoelectric forms of the complex that we observe. We plan to entertain the possibility that some of the variability is a result of variable patterns of apoprotein phosphorylation as is true of the chlorophyll a/b binding protein of some vascular plants .

Inventions

none

Publications

Roman S., N. Nadathur, E. Triplett, and B. Prezelin (1988) Light regulation of peridinin-chlorophyll a-protein complexes in the dinoflagellate Peridinium sp. use of anti-PCP antibodies to detect PCP gene products in cells grown in different light conditions. Plant Physiol. 88:594-599 (appendix 1)

Govind N., E. Triplett, S. Roman and B. Prezelin (1988) Characterization of the DNA from the Dinoflagellate Glenodinium sp.. Submitted to Journal of Phycology 9/1/88. Revised manuscript resubmitted 5/25/89. (Appendix 2)

Govind N., E. Triplett, S. Roman, S. Chang, and B. Prezelin (1988) cDNA clones encoding the peridinin-chlorophyll-protein (PCP) complex apoprotein from Glenodinium sp.: isolation and characterization (manuscript prepared for submission to Gene (appendix 3)

Roman S., E. Triplett and B. Prezelin (1988) Use of anti-PCP antibodies to quantify PCP apoproteins and to indicate changes in mRNA species in Glenodinium sp. grown under different light conditions. Presented at the Ocean Sciences Meeting (AGU/ASLO), New Orleans, LA. (appendix 4)

Chang. S, S. Govind, E. Triplett and B. Prezelin (1988). Molecular biology of the dinoflagellate Glenodinium sp.: initial isolation and characterization of cDNA clones encoding the apoprotein of peridin- chlorophyll a-protein complexes. Presented at the Ocean Sciences Meeting (AGU/ASLO), New Orleans, LA.

Govind, N., S. Chang, S. Roman, E. Triplett and B. Prezelin (1988). Molecular biology of the marine dinoflagellate Glenodinium sp.: Use of anti-PCP antibodies to isolate and characterize cDNAs encoding the apoprotein of peridin-chlorophyll a-protein complexes. Presented at the "Chromophyte Algae" Meeting, Plymouth England.(Appendix 5)

Nelson N. and B. Prezelin (1988) Light induced variations in specific absorption, pigmentation, and wavelength-dependent package effect in the dinoflagellate Glenodinium sp. American Society for Limnology and Oceanography. San Francisco, December 1988. EOS 69(44):1107-1108 (appendix 6)

Roman S., N. Govind, E. Triplett, R.Trench, B. Sweeney and B. Prezelin (1988) Specificity of Immunological cross-reactivity of anti-PCP antibodies with light harvesting peridinin-chlorophyll protein complexes purified from free-living and symbiotic dinoflagellates. Presented at the San Francisco winter meeting of

the American Society of Limnology and Oceanography (ASLO) EOS 69 (44):1099 (Appendix 7)

Invited lectures

Prezelin, B. A week long visit as a distinguished Visiting lecturer at Univ. of Stonybrook, New York. Several aspects of research in areas of the molecular biology, photophysiology and optical modeling of marine primary production were presented in four tutorial lectures and numerous small working groups with faculty and students

Prezelin B. "Application of Biotechnological and Molecular Biology Tools to the study of Optical Biology of Marine Photosynthesis." Workshop on the transfer of the tools of Biotechnology and Molecular Biology to the Ocean Sciences; Tuscon, Arizona; Sept 16-18, 1988

Training Activities

Dr. Govind Nadathur (a postdoctoral fellow), Norman Nelson and Raphael Jovine (graduate students), and Eric Brown and Bryan Strauss (undergraduates), are working on this project. Professor Beatrice Sweeney is working in our laboratory on a related Dinoflagellate project.

PhD thesis resulting from this project:

Roman S. (August 1988) Molecular Biology of the Marine Dinoflagellate Glenodinium 'sp.: Genome Characterization and light regulation of Gene Expression. University of California Santa Barbara.

Awards and Fellowships

Steven Roman, Raphael Jovine and Norman Nelson are recipients of University of California Biotech Training Grant Fellowships.

Minority Status

Dr. Nadathur is Indian